Osteogenic Inducible Small Molecule Compound Kit-6（Solution,With Alizarin Red S）

Cat: IK-OIN-6
Storage: -20℃,6 months.

Introduction

This Kit is designed by Solarbio specifically for the induction differentiation of osteoblasts. This package features 3 classic basic reagents and Alizarin Red S Stain Solution, all of which have good biological activity. It aims to build a "one-stop service platform" for customers, saving time, effort and worry. All the products in the library have passed biosafety testing and product quality testing, and have stable and effective performance, little difference between batches, and good biological activity. Moreover, there have been a lot of literatures and multi-party verification, and the quality is reliable.

Alizarin red S calcium staining is an authoritative and classical technique for the analysis of orange red calcium deposition in fixed cell samples by means of chelation technology, which makes calcium ions and alizarin red S produce complex. It is mainly suitable for the detection of calcium deposits and calcified nodules in animal protozoa or cultured cells. Under the influence of induction medium, stem cells will gradually differentiate into osteocytes, produce obvious calcium secretion reaction, and form calcium salt crystals or calcium nodules, which can be stained by alizarin red. There are orange or orange red deposits in the calcium salts, and the intensity of the color depends on the calcium salt content.

Kit Components

Reagent	Size	Storage
Reagent 1:10mM Dexamethasone	50uL	-20℃
Reagent 2:25mg/mL Vitamin C	300uL*2	-20℃
Reagent 3:1M β-Glycerophosphate Sodium	1.5mL	-20℃
Reagent 4:Alizarin Red S Stain Solution	10mL	2-8℃

Note：

a. Reagent 1, 2, 3 are sterile solutions, can be directly prepared into a complete medium for use.

b. Reagents 4 is the subsequent independent verification test components, do not mix with the medium.

c. Vitamin c is more unstable after dissolution, it is recommended to separate frozen storage (to avoid repeated freezing and thawing), in each liquid change, take out a thaw can be used. An additional reagent 2 is included with this Kit.

d. Reagent 4 can be stored at 2-8℃ away from light for 6 months.

e. This product is only used for scientific research experiments, not for clinical treatment.

Product Characteristics

1. This kit is a ready-to-use product, customers do not need to carry out complicated dissolution, sterilization, packaging and other steps, can be used directly.
2. Flexible customization. Customers can flexibly add or remove compounds (such as Alizarin Red, CPC, antibiotic, etc.) according to experimental needs. Customization of specifications and packaging is also possible.
3. Adequate inventory, spot delivery, high cost-effectiveness.

Protocols (*only for reference*)

1. Osteogenic Induction Differentiation (6-well plate as an example)

1.1(optional) Gelatin Coated Culture Vessel

After the stem cell culture for a long time, it may appear to peel off the wall or float phenomenon, it is recommended to use 0.1% gelatin solution on the incubator. The dish is coated. Prepare a suitable culture vessel, take an appropriate amount of gelatin to cover the bottom, stand at 37°C for 30 min, absorb and dry before use.

1.2 Stem Cell Inoculation

The cells of logarithmic growth stage were inoculated into the coated culture vessel according to the cell density of 2×10^4cells/cm^2, and cultured at 37℃ and 5% CO_2 until the fusion degree was 60-70%. The supernant was discarded, and 2mL osteogenic induction differentiation medium (containing dexamethasone, vitamin C and β-Glycerophosphate Sodium) was added to each well.

1.3 Induction of Cell Differentiation

The cells were cultured at 37℃ and 5% CO_2 for about 14 to 28 days, and the fluid was changed every 2 to 3 days, and the morphological changes of cells were observed. According to the precipitation of calcium salts and the formation of calcium nodules, the time of termination of cell induction was determined and staining was performed.

Note: If the curling is serious, half-change solution (only 1mL of fresh osteogenic induction differentiation medium is added each time) or half-change solution in the early stage and the late stage can be used for induction.

2. Dyeing Identification (6-well plate as an example)

2.1 Cell Fixation

Remove the culture medium and wash it once with an appropriate amount of 1×PBS. After discarding, take an appropriate amount of 4% neutral formaldehyde solution and cover the bottom surface of the culture vessel. Fixed at temperature for 30-60 min, discard the fixing solution, and then use 1×PBS to clean twice.

2.2 Alizarin Red Staining

Add alizarin red dye to each well for 3~5min, absorb the dye, wash twice with 1×PBS, and add an appropriate amount of 1×PBS to avoid cell drying.

Note: Dyeing time should not be too long, too long is easy to lead to calcium salt dispersion and poor coloring effect.

2.3 Induction Evaluation

The effect of osteogenic staining was observed under microscope, and image collection and induction evaluation were performed. Upon successful induction, the calcic nodules appear red or orange-red after binding with alizarin red dye.

Note: The level of osteogenic differentiation of stem cells varies depending on cell type, cell donor source, culture conditions, cell generation, cell state, and differentiation time.

Related Literature

[1]. Zhang Z, et al. A drug-loaded composite coating to improve osteogenic and antibacterial properties of Zn-1Mg porous scaffolds as biodegradable bone implants. Bioact Mater. 2023 Apr 28;27:488-504.(IF: 18.9)

Related Products

IK-OIN-1 Osteogenic Inducible Small Molecule Compound Kit-1
IK-OIN-2 Osteogenic Inducible Small Molecule Compound Kit-2
IK-OIN-3 Osteogenic Inducible Small Molecule Compound Kit-3
IK-OIN-4 Osteogenic Inducible Small Molecule Compound Kit-4
IK-LIN-1 Lipogenic Induction Of Small Molecule Compound Kit
IK-CEA-1 Cell Activation Kit-1